

THE ANATOMY AND HISTOLOGY OF THE LARVAL
ALIMENTARY CANAL OF THE EUROPEAN
CHAFER, AMPHIMALLON MAJALIS
RAZÓUMOWSKY (SCARABAEIDAE)¹

BY JAMES H. MENEES

DEPARTMENT OF ENTOMOLOGY AND LIMNOLOGY
CORNELL UNIVERSITY

The larvae of the European chafer, *Amphimallon majalis*, are serious pests of lawns, nursery stock and crops in the vicinity of Wayne county and other areas of northwestern New York. No studies have been made on the histology of this economically important grub. The following study describes the anatomy and histology of the alimentary canal of the third-instar larva, the most destructive stage in the life history of the chafer.

Third-instar larvae were fixed in Bouins fixative. Gross anatomical dissections were made in 70% ethyl alcohol, and histological studies were made from serial sections cut at 10 microns and stained with eosin and hematoxylin.

The morphology and musculature of the larval mouthparts of *A. majalis* and how they function in feeding has been described by Butt (1944); and Gyrisco et al. (1954) have described the life history of this beetle. Areekul (1957) has completed the most recent study of the comparative internal anatomy of several genera of larval Scarabaeidae; Wildbolz (1954) has worked on the anatomy and histology of the alimentary canal of the larva of *Melolontha melolontha*, L.; Schäfer (1954) has worked on *Rhizotrogus aestivus*; Subklew (1938) described the larval morphology of *Melolontha hippocastini*, but did not include studies on internal anatomy; Patterson (1937) studied the larval and adult digestive tracts of *Passalus cornutus*; Malouf (1932) described the anatomy of the head, stomodaeum and nervous system of the grub of *Pentodon dispar*; Fletcher (1930) studied the alimentary canal of the adult of *Phyllophaga gracilis*; Swingle (1930) has described the alimentary canal of the adult Japanese beetle, and Rittershaus (1927) described the alimentary canals of *Phyllopertha horticola* and *Anomala aenea*.

¹ The Grace H. Griswold fund is acknowledged for payment of plates.

The alimentary canal (Figs. 1 & 2) of the larval third-instar European chafer is approximately 30 mm. long and is slightly longer than the length of the larval body. Three divisions of the canal are easily recognized: the stomodaeum, ventriculus and proctodaeum. The stomodaeum and proctodaeum are ectodermal derivatives of the embryonic blastoderm while the ventriculus, or mesenteron, is an endodermal derivative. However, Johannsen and Butt (1941) state that in the Coleoptera, development of the mid-gut epithelium from the entoderm as well as from the ectoderm has been described.

The stomodaeum, extending from the mouth opening to the mesothoracic segment, is approximately 3 mm. long and is the shortest division of the alimentary canal. The stomodaeum consists of five areas: the buccal cavity, pharynx, esophagus, crop and esophageal valve.

The buccal cavity (Fig. 2, bc), is approximately 0.4 mm. in diameter and appears as an anterior dilation of the stomodaeum. Immediately posterior to the buccal cavity is the pharynx (ph), and posterior to the pharynx is the esophagus (es) which continues as a narrow tube approximately 1 mm. in length and 0.2 mm. in diameter. Posteriorly, the esophagus joins the crop. The crop (cr) is approximately 0.4 mm. in diameter, and appears as a posterior dilation of the stomodaeum. Generally, the crop has the characteristic function of food storage, as in the grasshopper, but the crop in the larval chafer appears to have lost this function almost completely, and the ventriculus appears to have taken over the greater part of food storage. The esophageal valve (Fig. 4, esv) is oval and approximately 0.6 mm. in diameter. It extends from the posterior region of the crop into the anterior end of the ventriculus where numerous folds of stomodaeal intima and epithelium project into the lumen of the ventriculus. This arrangement prevents to some degree the flow-back of food particles into the stomodaeum.

The ventriculus (Figs. 1 & 2, ven) is a large, round tube which extends from the mesothoracic segment to the 7th abdominal segment. The ventriculus is approximately 13 mm. long and 3-5 mm. wide and is the largest and longest part of the alimentary canal. The anterior limit of the ventriculus is defined by the esophageal valve and the posterior limit by the pyloric valve.

Caecal diverticula, sometimes called gastric caeca, (Figs. 1 & 2, acd, pcd) occur at the anterior and posterior areas of the ventriculus. There are ten anterior diverticula (Fig. 3, acd), eight being concentrically arranged around the anterior end of the ventricular-stomodaeal junction and two more are arranged within the the eight and below this junction. There are four posterior diverticula (Fig. 1, pcd) which lie ventrally on the ventriculus and immediately anterior to the external manifestation of the pyloric valve. In some dissections small rudimentary diverticula, four to six in number, were found on the mid-region of the ventriculus. Snodgrass (1935) says that caecal diverticula occur sometimes on parts of the ventriculus other than the anterior end surrounding the esophageal valve, and he figures three sets occurring in the scarabaeid larva of *Popillia japonica*. Rapp (1947) says that in the Scarabaeoidea the gastric caeca when present may number up to at least four sets, as in *Osmoderma eremicola*, and that the generally accepted belief that the gastric caeca are located at the anterior end of the midgut does not hold, since the gastric caeca in the Scarabaeoidea may be located on any portion of the ventriculus. It is apparent from this discussion that the number of caecal diverticula in larval insects may have taxonomic significance.

The proctodaeum is approximately 10 mm. long and consists of the anterior intestine (Fig. 2, ai), Malpighian tubules (mt), and the posterior intestine (pt), the latter being anatomically divided into the rectal sac (rs) and rectum (rt).

The anterior intestine is approximately 3 mm. long and has its greatest diameter, approximately 2 mm., where it joins the ventriculus. This large, funnel-shaped portion of the anterior intestine, sometimes called the proximal ileum, continues posteriorly to join the rectal sac.

The pyloric valve (Figs. 1 & 2, pv) is transversely concentric and has its most anterior articulation with the ventriculus on the ventral aspect of the alimentary canal near the posterior caecal diverticula. In a macroscopic sagittal section, the pyloric valve (Fig. 5, pv) appears as a circular swelling of ectodermal cells and intima which projects into the lumen of the canal.

There are four Malpighian tubules (Figs. 1 & 2, mt) and they are of the cryptonephridic type. Two tubules have their ampullar origins ventrally at the junction of the anterior intestine

with the ventriculus and two more have their origins laterally at this junction. These tubules extend anteriorly along the ventriculus to the area of the anterior caecal diverticula where they bend and extend posteriorly to terminate in a membrane on the ventral surface of the rectal sac (see Fig. 1). At their point of termination with the rectal sac, the tubules become intimately convoluted and entwined with one another to form a mesh-like network which is held in place by an enveloping membrane. Marcus (1938) says that in only a small number of the Coleoptera do the Malpighian tubules end freely in the body cavity, and Wigglesworth (1956) says that in many beetles the upper parts of the Malpighian tubules closely invest the rectum, being bound to it by a delicate membrane. Wigglesworth says further that this arrangement probably serves to add the absorptive powers of the Malpighian tubules to those of the rectal epithelium and that this arrangement seems always to be associated with remarkable powers of drying the excrement. Patton and Craig (1939) who studied the physiology and absorption of the cryptonephridic tubules of the mealworm, *Tenebrio molitor*, conclude that Malpighian tubes of this type serve only to absorb materials from the haemolymph, the entire reabsorption of water and utilizable materials taking place in the walls of the rectum.

In the chafer larva the excrement contained in the rectal sac is conspicuously dry and hard, and it seems to adhere to the thin intima of the rectal sac since it is very difficult to clean away. This undoubtedly indicates that the cells of the rectal sac play an active role in the reabsorption of water and utilizable food materials contained in the rectal sac.

The posterior intestine (Fig. 2, pt) is U-shaped and extends from the eighth through the tenth abdominal segments. It consists of the rectal sac (rs), approximately 5 mm. long and 5 mm. wide, and the rectum, approximately 3 mm. long and 2 mm. wide.

HISTOLOGY OF THE ALIMENTARY CANAL

The cells of the stomodaeum (Fig. 6) are generally cuboidal, but in the area of the esophageal valve (Fig. 9, esv) they appear columnar. Some of these cells and their intima are thrown into longitudinal folds which project into the lumen of the ventriculus, there being approximately six such folds. Such an arrange-

ment allows for the expansion of the stomodaeum while the larva ingests food.

The intima of the stomodaeum appears to be composed of primary and secondary intima. The primary intima (Fig. 6. pi) is continuous with the body wall and stains dark with hematoxylin. The secondary intima (si) is much thicker than the primary intima and appears as thin, almost transparent protoplasmic strands or fibrillae which are being secreted by the epithelium of the stomodaeum. The secondary intima stains light with hematoxylin, and such a staining reaction may suggest that the secondary intima being secreted is weakly acidic in comparison with the basic staining reaction of the cytoplasm with alcoholic eosin, and in comparison with the acidic staining reaction of the nucleus with hematoxylin.

The cells of the ventriculus (Fig. 11) consist of large columnar epithelium with a striated border (sb). The height of these cells is approximately 81 micra and the width approximately 18 micra. The nuclei are oval, finely granular and measure approximately 7.2 micra wide and 18 micra tall.

The striated border is irregular and is approximately 1.5 micra wide. Day and Waterhouse (1953) state that only the conspicuous nature of the striated border has resulted in its being considered anything but a normal cell specialization. Furthermore, they state that the striated border appears to occur in cells in which unusually active transfer occurs across the cell boundary. The fact that the striated border does not possess the motile characteristics of cilia suggest that it probably is made up of minute protoplasmic fibrillae. Maximow and Bloom (1955) state that in all vertebrates the cells of the simple columnar intestinal epithelium have a distinct layer of modified protoplasm, the striated border, on their free surface. They say further "electron microscopy fails to demonstrate a cement like substance, although the rod-like processes are clearly seen. Presumably, it (striated border) plays an important role in the absorption of nutritive substances from the intestinal cavity." It is clearly evident from the above discussion that the striated border of the ventricular epithelium of insects is strikingly similar both cytologically and functionally to the striated border of vertebrate epithelial cells. Further studies with the electron microscope on the striated border of the ventricular epithelium in the larval chafer are anticipated by this author.

The basal region of the ventricular epithelium is irregular and a basement membrane (bm) approximately 0.7 micra wide is evident. The nidi (ni), or regenerative cells, lie irregularly in groups of four–six near the basement membrane. The nidi stain conspicuously darker with hematoxylin than do the nuclei of the columnar cells. Wildbolz (1954) states that the middle intestine of *Melolontha melolontha* possesses two morphologically different regions; he says that in the anterior region of the ventriculus the epithelium has regularly arranged crypts and the regenerative cells lie at the base of the crypts, while in the posterior region of the ventriculus the epithelium is even and only a few regenerative cells are found. Areekul (1957) has found that the replacement cells of the ventriculus in several scarabaeid larvae have 3–4 nuclei in each group, and he apparently found no morphologically different regions in the ventriculus. Areekul says further that the structure of the gastric caeca does not differ from that of the ventriculus except that the number of epithelial cells seems to increase tremendously at times.

In the anterior and posterior regions of the ventriculus of *A. majalis*, the nidi are not found in crypts, and the epithelial cells in all regions of the ventriculus are even. In the anterior region of the ventriculus near the anterior caecal diverticula crypts may be found, but these crypts are not regularly arranged nor do the nidi appear to be found in a regular fashion at the bases of these crypts.

A thin peritrophic membrane (Fig. 9, pm) is present and envelops the food contents of the ventriculus. This membrane protects the epithelial cells of the ventriculus from ingested food stuffs and is permeable to digestive enzymes and to the products of digestion. Day and Waterhouse state that two types of peritrophic membranes occur in insects and that two methods of formation have been described. The first type is a single, uniformly continuous structure which is produced as a viscous secretion by a ring of cells at the junction of the stomodaeal and ventricular epithelium (see Butt, 1934). The second type is a tube consisting of a series of concentric lamellae and is thought to be produced by all the epithelial cells of the ventriculus secreting a series of thin lamellae. Snodgrass (1935) states that in the formation of the second type there is no reason for supposing

that chitin could not be produced from endodermal (ventriculus) as well as from ectodermal derivatives (stomodaeum) of the blastoderm. Snodgrass says further that the second type of peritrophic membrane is evidently to be regarded as a chitinous intima of the ventriculus.

Intracellular protozoans (Fig. 11, p) were noticed in the distal ends of many cells of the ventricular epithelium. The nuclei of these protozoans are coarsely granular and are slightly smaller than the nuclei of the columnar cells. The nucleoplasm of the protozoans stains darker with hematoxylin than do the nuclei of the columnar cells, and the cytoplasm of the protozoans appears to stain similarly to that of the columnar cells. A clear, hyaline area is generally found around the entire protozoan. These protozoans are quite numerous in the epithelium of the ventriculus and approximately 3–4 were found for each ten cells observed. It is difficult to say at present what type of intracellular protozoan this may be and if it is a parasite or a symbiont. Further studies are being made in order to classify the complete protozoan fauna of this larval beetle.

The cells of the anterior intestine (Fig. 7) are cuboidal and elongated cuboidal. Cell boundaries are, in general, not clearly distinguished and cells appear as a syncytium. The nuclei (n) are oval and are approximately 10.8 micra long and 7.2 micra wide. In the region of the pyloric valve (Fig. 10, pv) the cells attain columnar form with their nuclei lying near the distal end of the cells.

Longitudinal folds of epithelium and intima similar to those of the stomodaeum project likewise into the lumen of the anterior intestine. The intima of the anterior intestine is also composed of primary and secondary intima. The thickness of the primary intima varies from 1.8 to 3.6 micra.

The rectal sac (Fig. 12) is made up of contiguous and alternating areas of elongated cuboidal cells and hillocks of near columnar cells. The epithelial wall, in cross-section, appears much thinner than the cell wall of the anterior intestine. The nuclei of these cells are rounder than the nuclei of the anterior intestine. The elongated cuboidal cells are approximately 21 micra long and 9 micra high. The near columnar cells are approximately 40 micra high and 15 micra wide. A basement membrane (bm) is usually evident and describes an irregular

course about the basal region of the cells. The muscularis of the rectal sac is similar to that of the anterior intestine, but there is a reduction in the number of muscles.

The cells of the rectum (Fig. 13) are taller and wider than those of the anterior intestine and rectal sac. They are approximately 60 micra high and 20 micra wide. These cells appear to be irregularly cuboidal and columnar. The nuclei are oval, lie near the basal end of the cell and are approximately 12 micra tall and 7 micra wide. The longitudinal folds of the rectum are similar to those of the anterior intestine, but they are not as numerous nor do they project as far into the lumen of the rectum as do the folds of the anterior intestine.

The cells of the Malpighian tubules (Fig. 8) are cuboidal and appear to give off secretions of the merocrine type. The nuclei of these cells are oval and are approximately 13 micra in diameter. A basement membrane (bm) is evident and a small striated border (sb) similar to that found in the ventricular epithelial cells is also evident. A nucleated peritoneal sheath (pms) surrounds the tubules and is approximately 4.3 micra thick.

SUMMARY

The alimentary canal of the larval European chafer consists of three anatomically and histologically differentiated areas—the stomodaeum, ventriculus and proctodaeum; the stomodaeum and proctodaeum are ectodermal derivatives of the embryonic blastoderm, while the ventriculus is an endodermal derivative. The stomodaeum consists of the buccal cavity, pharynx, esophagus, and much reduced crop, while the proctodaeum consists of the anterior intestine, rectal sac, and rectum.

The histology of the stomodaeum and proctodaeum is similar, consisting of cuboidal epithelium which secretes a chitinous intima. The epithelium and intima of the stomodaeum and proctodaeum are thrown into approximately six folds which project into the lumen of the canal. This allows for expansion as greater amounts of food are ingested or excreted.

The cells of the rectum appear to be active in the reabsorption of water and utilizable food materials from within the rectal sac.

The Malpighian tubules are of the cryptonephridic type and the tubules may also play an active part in the absorption of water and materials from the rectal sac.

The ventriculus contains two sets of caecal diverticula, ten anterior and four posterior. These may be of some taxonomic value. The histology of the ventriculus appears to be uniform in that it consists of columnar epithelial cells with a striated border. The striated border appears to be analogous both in structure and function to the striated border of vertebrate epithelial cells. The nidi are irregularly arranged along the bases of the ventricular epithelium and they stain darker with hematoxylin than do the nuclei of the ventricular epithelial cells.

LITERATURE CITED

- AREEKUL, S. 1957. The comparative internal larval anatomy of several genera of Scarabaeidae (Coleoptera). *Ann. Ent. Soc. Amer.* **50**: 562-577, 59 figs.
- BUTT, F. H. 1934. The origin of the peritrophic membrane in *Sciara* and the honey bee. *Psyche*. **41**: 51-56, 1 plate.
- BUTT, F. H. 1944. External morphology of *Amphimallon majalis*. Cornell Univ. Exper. Sta. Memoir no. 266. 18 pp., 13 plates.
- DAY, M. F. AND D. F. WATERHOUSE. 1953. Structure of the alimentary canal. In Roeder, *Insect Physiology*. New York, John Wiley and Sons. pp. 273-298.
- FLETCHER, F. W. 1930. The alimentary canal of *Phyllophaga gracilis*. *Ohio Journ. Sci.* **30**: 109-119.
- GYRISCO, G., R. WHITCOMB, R. BURRAGE, C. LOGOTHETIS AND H. SCHWARDT. 1954. Biology of the European chafer, *Amphimallon majalis*. Cornell Univ. Agric. Exp. Sta. Memoir no. 328, 35 pp.
- MALOUF, N. 1932. The morphology of the head of a white grub. *Bull. Soc. Royal Entom. Egypt.* pp. 66-88, 37 figs.
- MARCUS, B. A. 1930. Untersuchungen über die Malpighischen Gefässe bei Käfern. *Zeit. Morph. Ökol. Tiere.* **19**: 609-677.
- MAXIMOW, A. AND W. BLOOM. 1955. A textbook of Histology. W. B. Saunders Co., Philadelphia and London, 616 pages.
- PATTERSON, M. 1937. The cellular structure of the digestive tract of the beetle *Passalus cornutus*. *Ann. Ent. Soc. Amer.* **30**: 619-640, 5 plates.
- PATTON, R. AND R. CRAIG. 1939. The rates of excretion of certain substances by the larvae of the mealworm, *Tenebrio molitor*. *Jour. Exp. Zool.* **81**: 437-456.
- RAPP, W. F. 1947. The number of gastric caeca in some larval Scarabaeoidea. *Canadian Entomologist.* **79** (5): pp. 145-147.
- RITTERSHAUS, K. 1927. Studien zur Morphologie und Biologie von *Phyllopertha horticola* L. und *Anomala aenea*, G. (Coleoptera). *Zeit. Morph. Okol. Tiere.* **8**: 271-408.
- SCHÄFER, R. S. 1954. Zur Kenntnis der Anatomie, Physiologie und Oekologie des Brachkäfers, *Rhizotrogus aestivus* Oliv. (Col. Lam.). *Zeit. Angewandte Entom.* **35**: 381-424.

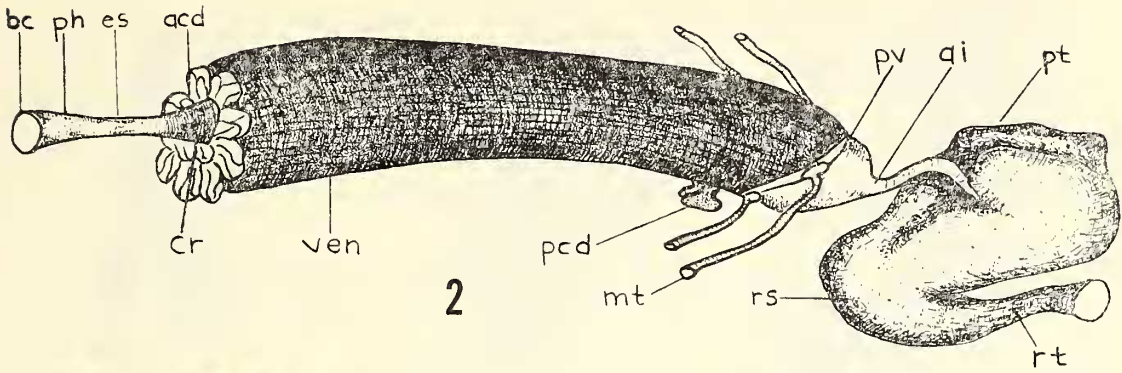
- SNODGRASS, R. E. 1935. Principles of Insect Morphology. McGraw-Hill Book Co. New York. 667 pages.
- SUBKLEW, W. 1938. Zur Morphologie der Larve von *Melolontha hippocastani*. Archiv. Naturgesch. 7: 270-304, 24 figs.
- SWINGLE, M. C. 1930. Anatomy and physiology of the digestive tract of the Japanese beetle. Jour. Agric. Research. 41: 181-196, 4 figs.
- WILDBOLZ, T. 1954. Beitrag zur Anatomie, Histologie und Physiologie des Darmkanals der Larve von *Melolontha melolontha*. Mitt. Schweiz. ent. Ges. Berne. 27: 193-240, 25 figs.
- WIGGLESWORTH, V. B. 1956. Insect physiology. London: Methuen and Co., Ltd. New York: John Wiley and Sons, Inc. 130 pages.

ABBREVIATIONS

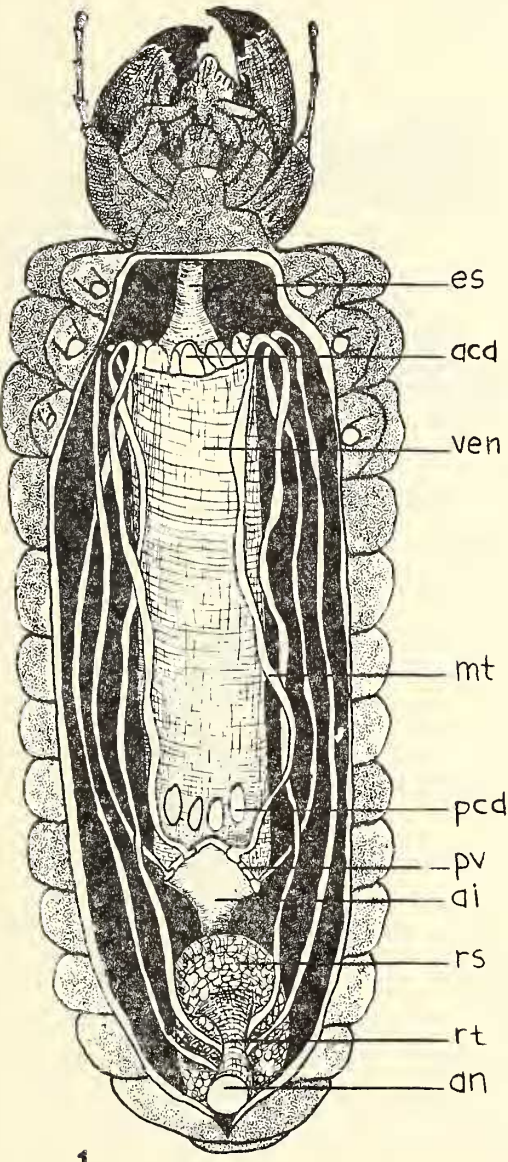
acd—anterior caecal diverticula	p —protozoan
ai —anterior intestine	pcd —posterior caecal diverticula
an —anus	ph —pharynx
bc —buccal cavity	pi —primary intima
bm —basement membrane	pm —peritrophic membrane
cm —circular muscles	pms—peritoneal sheath
cr —crop	pt —posterior intestine
es —esophagus	pv —pyloric valve
esv —esophageal valve	rs —rectal sac
in —intima	rt —rectum
lm —longitudinal muscles	sb —striated border
mt —Malpighian tubules	si —secondary intima
n —nucleus	ven —ventriculus
ni —nidi	

EXPLANATION OF FIGURES

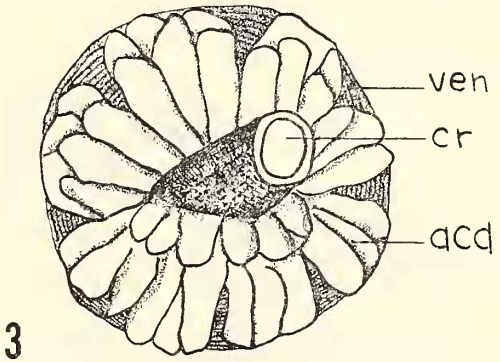
Fig. 1—ventral dissection of third-instar larva showing ventral aspect of alimentary canal; Fig. 2—lateral aspect of complete alimentary canal; Fig. 3—external frontal aspect of ventriculus showing anterior caecal diverticula; Fig. 4—internal frontal aspect of ventriculus showing esophageal valve; Fig. 5—macroscopic sagittal section of ventriculus and anterior intestine showing pyloric valve; Fig. 6—cross-section of stomodaeum; Fig. 7—cross-section of anterior intestine; Fig. 8—longitudinal section of Malpighian tubule; Fig. 9—sagittal section of stomodaeum and ventriculus showing esophageal valve; Fig. 10—sagittal section of ventriculus and anterior intestine showing pyloric valve; Fig. 11—cross-section of ventriculus; Fig. 12—cross-section of rectal sac; Fig. 13—cross-section of rectum.



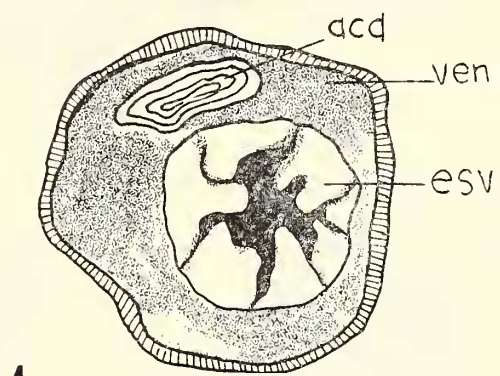
2



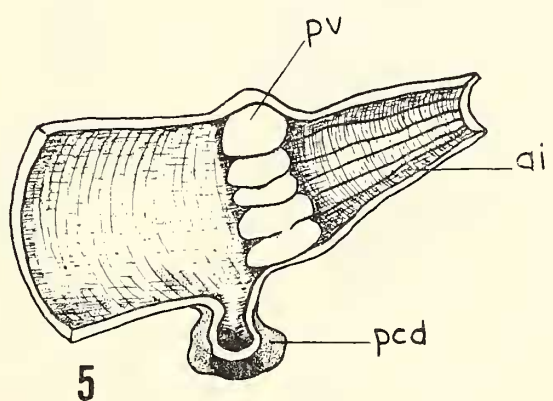
1



3



4



5

